THE NUCLEOTIDE SEQUENCES OF TWO THREONINE tRNAs FROM BREWER's YEAST

Jean WEISSENBACH, István KIRÁLY and Guy DIRHEIMER

Laboratoire de Biochimie, Institut de Biologie Moléculaire et Cellulaire du CNRS, 15 rue Descartes, 67084 Strasbourg Cedex and *Laboratoire de Toxicologie et de Biologie Moléculaires, Faculté de Pharmacie, Université Louis Pasteur, 67083 Strasbourg Cedex, France*

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1. Introduction

The nucleotide sequence of threonine tRNA from *E. coli* has been determined [1]. This tRNA is charged with the heterologous yeast threonyl-tRNA synthetase [2]. Reciprocally, yeast threonine tRNA is aminoacylated to a high extent with the *E. coli* threonyl-tRNA synthetase. It was therefore interesting to determine the primary structure of yeast $tRNA^{Thr}$. We present here the primary structure of the major $tRNAs^{Thr}$ of brewer's yeast.

2. Materials and methods

2.1. *Preparation of purified $tRNA_1^{Thr}$*

Brewer's yeast $tRNA_1^{Thr}$ was prepared by countercurrent distribution [3] followed by the chromatography of the most active fractions on Sepharose 4B at pH 4.5 using a reverse ammonium sulphate gradient [4] from 2 M to 1 M at 20°C. $tRNA_1^{Thr}$ eluted in a sharp peak and had an average accepting capacity of 1500 pmol threonine per A_{260} unit.

2.2. *Sequencing techniques*

The methods of exhaustive enzymatic and alkaline digestions of tRNA or of oligonucleotides and the techniques of oligonucleotide fractionation have been previously described [5–7]. Partial T_1 ribonuclease digestion was performed by incubation of 375 A_{260} units of purified $tRNA_1^{Thr}$ with 3000 units of RNase T_1 in 5 ml of a 0.1 M Tris–HCl buffer (pH 7.5) for 30 min at 0°C.

By overlapping the larger fragments and by assuming that t^6A in threonine tRNA occupies the same position as in all tRNAs recognizing a codon starting with A [8,9] it has been possible to derive the linear order of the nucleotides of $tRNAs_1^{Thr}$ shown in fig.1.

3. Results and discussion

The results of the analysis showed that the $tRNA_1^{Thr}$ we isolated occurs as two nucleotide sequences represented in about equimolar amounts. In one species ($tRNA_{1b}^{Thr}$) a C·G base pair in the TψC stem replaces the A·U base pair of the other species

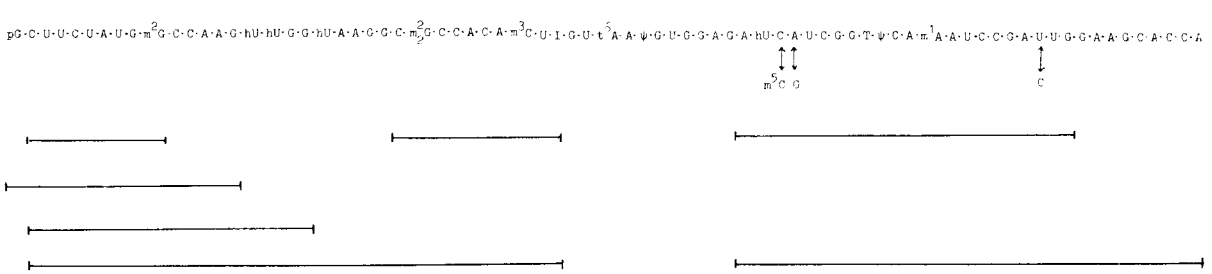

Fig.1. Summary of overlaps which were obtained by partial digestion of brewer's yeast $tRNAs_1^{Thr}$ with T_1 RNase.

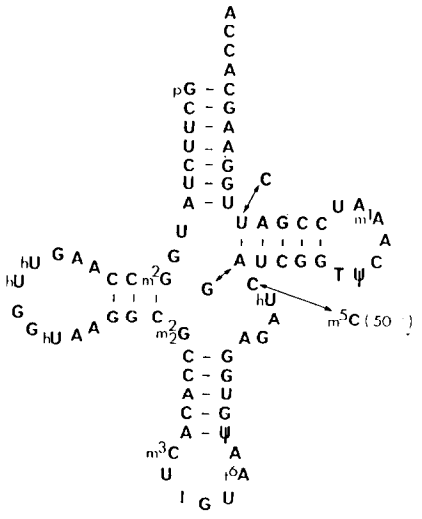

Fig.2. Cloverleaf model of brewer's yeast $tRNAs_1^{Thr}$. The tRNA contains 76 nucleosides including the modified nucleosides: 1-methyladenosine (m^1A), *N*-9 (β-D-ribofuranosyl)-purine-6-yl-carbamoyl threonine (t^6A), 3-methylcytidine (m^3C), 5-methylcytidine (m^5C), 2-methylguanosine (m^2G), 2,2-dimethylguanosine (m_2^2G), inosine (I), 5,6-dihydrouridine (hU), pseudouridine (ψ), and ribothymidine (T).

($tRNA_{1a}^{Thr}$) as indicated (fig.2). It is probable that structural reasons favoured the conservation of the Watson–Crick base pairs.

The two $tRNAs_1^{Thr}$ are composed of 76 nucleotide residues including 14 minor nucleotides (fig.2).

Noteworthy is the presence of the very rare nucleoside 3-methyl-cytidine which is not present in any of the known yeast tRNA structures but has been found in the same position in rat liver serine tRNAs [10,11] and has been described in total yeast tRNA [12]. The nucleoside t^6A has always been found in tRNAs recognising codons starting with A [8,9] which is also the case for $tRNAs_1^{Thr}$, the codons for threonine being A–C–N. In *E. coli* $tRNA^{Thr}$ this nucleotide is the *N*-methyl derivative of t^6A but in yeast $tRNA_1^{Thr}$ we only found the non methylated t^6A. The nucleoside C of the extra arm is only incompletely modified (50%) to m^5C in both species of $tRNAs_1^{Thr}$.

The anticodon of the yeast $tRNAs_1^{Thr}$ is I–G–U. According to the wobble hypothesis it could recognise [13] 3 of the 4 codons for threonine: A–C–U, A–C–C and A–C–A. The 4th triplet A–C–G might be recognised by a second isoaccepting threonine tRNA which appears in the counter-current distribution profiles of brewer's yeast tRNA [3].

A comparison of threonine tRNA from *E. coli* with yeast $tRNAs_1^{Thr}$ (fig.3) shows that either 26 (in $tRNA_{1a}^{Thr}$) or 24 ($tRNA_{1b}^{Thr}$) out of 76 nucleotides are different if we do not take into account the post-transcriptional modifications of the bases. These differences involve five altered bases in the amino acid acceptor stem, three altered base pairs in the anticodon stem, two or three base pairs in the TψC stem, one in the hU stem and a base in the extra arm. The largest common sequence is 8 nucleotides long and represents almost entirely the hU loop. The 5′-terminal sequence pG–C–U and the 3′-terminal sequence A–G–C–A–C–C–A are also identical but they are also found in the noninitiating brewer's yeast methionine tRNA [14]. This observation is not consistent with 'The Acceptor Stem Hypothesis' proposed by Schulman and Chambers [15] who postulated that the first three base pairs of the amino acid stem function as the specific recognition site for the aminoacyl-tRNA synthetases.

The comparison of the primary structures of the *E. coli* and yeast $tRNA^{Thr}$ both cross charged with the heterologous enzymes does not give information

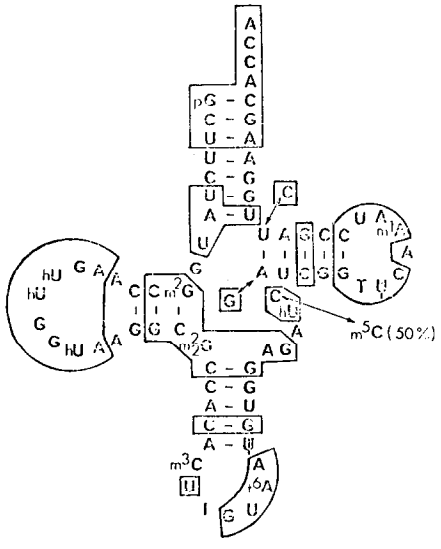

Fig.3. Comparison of the primary structure of brewer's yeast and *E. coli* $tRNA^{Thr}$. Boxes: Homologies between the two tRNAs. The differences in the state of post-transcriptional modifications were disregarded in comparison.

about the specific recognition sites. Therefore we think that with these tRNAs, as well as with others previously studied [16] the tertiary structure of the tRNAs rather than common sequences leads to a precise positioning of some functional groups ($-NH_2$, $-OH$, $-PO_3^{2-}$) thus conditioning the kinetic parameters responsible for the specific amino-acylation of the tRNAs [17].

Acknowledgements

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